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THE RHIZOSPHERE EFFECT OF MYCORRHIZAL AND NON-MYCORRHIZAL ROOTS OF YELLOW BIRCH SEEDLINGS[1]

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Abstract

A study of the surface microflora of mycorrhizal and non-mycorrhizal roots of yellow birch seedlings has revealed distinct quantitative and qualitative differences. Higher bacterial counts and distinctly greater numbers of actinomycetes and of methylene-blue-reducing, sugar-fermenting, ammonifying, and fluorescent-pigment-producing bacteria were found on the former. The percentage incidence of bacteria growing optimally in a simple mineral–glucose medium was greater on normal roots whereas bacteria requiring complex nutritional factors appeared to be relatively more numerous on the mycorrhizal surface. Species of *Pythium*, *Fusarium*, and *Cylindrocarpon* predominated on, and *Mycelium radicis* (?) was absent from, normal roots, whereas, on mycorrhizal roots, *Penicillium* and other rapidly growing fungi as well as *Mycelium radicis* (?) were numerous and *Pythium* and *Fusarium* species absent. These results are discussed in relation to the nutrition of mycorrhizal and non-mycorrhizal roots.

Introduction

The growth of a root in soil is accompanied by extensive microbial activity at the root–soil interface (the rhizosphere effect). This is due primarily to the liberation by the root of organic and inorganic nutrients which are readily consumed by the organisms in its vicinity; sloughed-off dead and dying apical and epidermal tissue, root hairs, and other root parts also contribute to this food supply. The rhizosphere effect has been demonstrated with a wide variety of crop plants and trees (5, 10, 15). It is influenced by many factors, such as the kind and stage of development of the plant; the type, treatment, and moisture content of the soil; and environmental conditions such as temperature and light (10, 14, 15). These factors may act directly on the soil microflora and (or) indirectly by influencing plant growth.

The penetration of roots by fungi resulting in the establishment of mycorrhizal structures (4) may be considered as a special rhizosphere phenomenon, perhaps analogous to the production of nodules by the legume bacteria. The modification of the root by the fungus might be expected to alter, among other things, the nature and amount of root excretions, thereby affecting the numbers and types of organisms developing in the vicinity of, or on, the invaded root. As there is little published information on this subject, it was considered

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of interest to compare the microflora of mycorrhizal and non-mycorrhizal roots. The results of these experiments are reported in this paper.

Materials and Methods

Yellow birch seedlings were grown in a forest soil in a large rectangular tank in the greenhouse. After being carefully removed from the soil, the roots were washed in water and sections with typical ectotrophic mycorrhizae removed for further washing. This was done four times in sterile water and the roots suspended in sterile water in large Petri plates. Rootlets showing typical mycorrhizal structures and apparently normal rootlets of approximately the same size were removed separately with sterile forceps and transferred to 5 ml water in sterile hand homogenizers. Each sample was thoroughly ground to give a uniform suspension which was then suitably diluted for bacterial and fungal counts (11, 13). Qualitative studies of fungi were conducted as described by Peterson (13). Detached mycorrhizal and normal rootlets of similar size were subjected to serial washings and placed on agar medium for direct isolation of fungi. In all, 120 segments of each type of root were used. Extinction dilution procedures were used to estimate numbers of methylene-blue-reducing, sugar-fermenting, ammonifying, and fluorescing bacteria (1, 6, 8). The nutritional requirements of bacteria isolated from the plates used for counting were determined by the method of Lochhead and Chase (12).

The turbidity of suitable aliquots of the root homogenates was determined in a Klett–Summerson colorimeter (540 filter) and the samples dried at 80° C and weighed. A standard curve was then constructed which permitted rapid determination of the weight of very small amounts of homogenized material. This procedure was extremely useful as it required many hours of laborious work to obtain enough root material for microbial analysis. All the counts reported are calculated on an oven-dry basis.

Results

Representative data are shown in Table I. It appears that with the exception of the fungi, the soil microflora is stimulated more strongly by mycor-

TABLE I

Microbial numbers on mycorrhizal and normal roots of yellow birch seedlings*

Types of microorganisms	Mycorrhizal roots	Normal roots
Bacteria	3,930,000	2,650.000
Actinomycetes	3,000	340
Fungi	16	32
Methylene-blue-reducing bacteria	576,000	49,500
Glucose-fermenting bacteria		
Acid	45,500	495
Gas	57,600	10
Sucrose-fermenting bacteria†		
Acid	1,000	54
Gas	1,000	30
Fluorescent-pigment-producing bacteria	273	10
Ammonifying bacteria	121,000	13,000

*Thousands per gram oven-dry root material.
†Separate experiment.

rhizal roots than by normal roots. The relatively low fungal counts may be due to the method of preparing the samples for analysis, since homogenization may cause excessive damage to the mycelium of some fungi. A qualitative analysis of the colonies on the dilution plates used for fungal counts showed a preponderance of rapidly sporing types (*Penicillium*, *Trichoderma*) on both types of root material. As usual, bacteria are the most numerous organisms, with actinomycetes next, although as has been pointed out frequently, total numbers may not be a critical consideration since cell or mycelial mass may more than make up for numbers. The very high counts obtained may also be due to the method of calculation, based on dry weight of root material. Lower numbers would have been obtained if the counts were based on moist weight or surface area; this was not possible under the conditions of these experiments. A somewhat higher bacterial count was obtained with the mycorrhizal roots, the ratio of mycorrhizal to non-mycorrhizal numbers being 1.6:1. On repetition of this entire experiment, this ratio was found to be 1.5:1. Of much greater interest, however, are the tests for bacteria capable of accomplishing certain biochemical changes such as ammonia production, sugar fermentation, and methylene blue reduction. The methylene-blue-reducing and the ammonifying bacteria are 10 times more numerous on mycorrhizal than on normal roots. Bacteria producing acid and gas from glucose and sucrose and green fluorescent types are stimulated to an even greater extent on the mycorrhizal roots although their absolute numbers are lower than those of the ammonifying and methylene-blue-reducing forms.

The gross nutritional requirements of the bacteria isolated from both types of roots are given in Table II. By far the majority of these organisms required amino acids for optimal growth but there was no significant difference in the percentage incidence of these isolates on the two types of roots. The figures obtained are very similar to those found by Ivarson and Katznelson (5) in the rhizosphere of these seedlings. Noteworthy also is the preponderance of bacteria growing on a simple synthetic medium (B) on normal roots and the greater proportion (17%) of organisms with complex requirements on the mycorrhizal roots. Preliminary tests showed that there was a lower percentage incidence of rapidly growing bacteria as well as a smaller proportion of phosphate-solubilizing bacteria (7) on the mycorrhizal roots.

Table III illustrates the types of fungi isolated from segments of both types of roots. On normal roots, species of *Pythium*, *Fusarium*, and *Cylindrocarpon* predominate, while *Mycelium radicis* (?) is absent. On mycorrhizal roots, how-

TABLE II

Nutritional requirements of bacteria isolated from mycorrhizal and normal roots

Media	Mycorrhizal roots, %	Normal roots, %
B (minerals, glucose)	16	34
A (B + casamino acids)	67	65
YS (B + yeast and soil extracts)	6	1
No growth in YS medium	11	0
Number of cultures tested	81	96

ever, *Penicillium* and other rapidly sporing types preponderate; *Mycelium radicis* (?) is abundant; and *Pythium* and *Fusarium* species are absent.

TABLE III

Fungi isolated from washed root segments of yellow birch seedlings

Genera	Percentage incidence	
	Mycorrhizal roots	Normal roots
Pythium		14
Mortierella	3	
Emericellopsis		1
Phoma	2	9
Coniothyrium	2	
Trichoderma	4	
Aspergillus	2	
Penicillium	16	
Paecilomyces	8	
Gliocladium	2	
*Hyalostachybotrys**		8
Periconia	2	
*Rhinocladiella**		1
*Phialocephala**	7	
Fusarium		24
Cylindrocarpon	21	38
Myrothecium	1	
Mycelium (radicis)†	22	
Others	8	4
Fungus-free segments	0	37
Total numbers of cultures	100	91

*Kindly identified by Dr. S. J. Hughes, Plant Research Institute, Canada Department of Agriculture.
†Tentative identification.

Discussion

The results show clearly that mycorrhizal roots exert a stimulatory effect on numbers of certain physiological groups of soil bacteria and perhaps also on soil actinomycetes. Other types of soil bacteria, such as those growing optimally in a simple medium, appear to be repressed in this region. Fungal numbers are also lower on the mycorrhizal tips. Since such counts usually represent spore-forming types whose rapid growth frequently obscures more slowly developing organisms, the significance of numbers alone is questionable. This conclusion is borne out by the results in Table III, which show striking differences between the two types of roots. Significantly there is a complete absence of the mycorrhizal fungus *Mycelium radicis* (?) from the normal roots, yet this organism represents 22% of the isolates from the mycorrhizal root segments. The absence of heavily sporing forms from normal root segments and the large number of fungus-free normal segments (37%) indicate the effectiveness of the washing procedure in removing fungal spores. The presence of such types on mycorrhizal roots suggests either that their spores were enmeshed in the mycorrhizal net and could not be dislodged by washing or that they were present as mycelia growing in association with the mycorrhizal fungus. Harley (4) also states "that the fungi in the washings of roots

and on the unwashed roots are mainly sporing species" whereas sterile mycelia, sporing with difficulty, "comprise a significant proportion of colonies observed on the washed roots." It is likely that, in the experiments reported above, even larger numbers of the latter type would have been isolated were it not for overgrowth by *Penicillium* and other rapidly growing fungi.

The greater numbers of the various types of bacteria and of actinomycetes on the mycorrhizal roots suggests a greater availability of nutrients in this zone. These include oxidizable or fermentable substances, nitrogenous materials capable of being ammonified, a variety of growth factors, and inorganic substances that were made available through the solubilizing activity of the mycorrhizal fungus or that moved out of the living fungal hyphae as a normal process (2). The increase in absorbing surface associated with mycorrhizae (4) may also provide a greater area for excretion than is the case with normal roots. Since many of the mycorrhizal roots in the present tests were brownish in color, probably representing mature rather than young structures (4), it is conceivable that some autolysis or degeneration of the mycorrhizal mantle may have taken place. This would also result in the liberation of organic and inorganic nutrients with a concomitant stimulation of microbial development. It may be of some significance in this connection that 17% of the bacterial isolates from the mycorrhizal roots required complex (YS) or unknown factors for growth whereas 34% of the isolates from normal roots could grow optimally in a simple synthetic medium (Table II).

The increased bacterial population on the mycorrhizal roots may have a marked effect on the nutrient uptake by or through these structures. Whereas competition for nutrients must occur, there is no doubt that rhizosphere and rhizoplane organisms are responsible for many transformations leading to the release of nutrients and even more complex substances such as auxins which may be used by plants (3, 7, 9). This is particularly true in the rich humus layers in which mycorrhizae abound (4) and where, despite the rather low pH, many bacteria are also present (5). A case in point is the potential ammonifying capacity of the rhizosphere microflora which may result in the liberation of appreciable amounts of ammonia from the complex organic nitrogenous constituents of humus. The ammonia nitrogen could be utilized directly whereas, in the combined organic state, the nitrogen would not be readily available to the plant.

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